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Agronomic Performances of Low Linolenic Acid Soybean Mutant Lines Developed from Cultivar Bay

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Summary

Agronomic performances and oil content of six low linolenic acid soybean [*Glycine max* (L.) Merr.] genotypes (LOLL, MS382, MOLL, M-5, M24 and KL-8), and cultivar Bay were evaluated. Each line contains 2.7, 2.7, 3.7, 4.1, 5.8 and 6.5% linolenic acid respectively, while Bay has 7.6%. Experiment was conducted in a randomized complete block design with four replications. At maturity, agronomic traits, such as flowering time, maturity, plant height, number of branches, number of nodes, total pods, total seeds, seed yield, oil content and fatty acid compositions were analyzed, and effect of lowering linolenic acid on the other traits were investigated. Flowering time, maturity and oil content of these lines not showed any significant differences. Similar number of branches and nodes were observed in lines LOLL, MS382, M-5, M24 and Bay. Plant height and seed yield traits varied among the lines. M-5 and M24 showed comparable agronomic performances as Bay. In LOLL and MS382 little yield reductions were observed. Extremely poor agronomic performances were observed in KL-8 and MOLL (recombinant low linolenic acid line of KL-8 x M-5). It indicates that the mutation for low linolenic acid in KL-8 is associate with poor agronomic traits. In other lines, detrimental effect of mutation on agronomic traits were not observed. Phenotypic correlation coefficients of linolenic acid content with agronomic traits, seed yield and oil content shows that there are no significant correlations between them.

Key words: *Glycine max*, low linolenic acid mutant lines, agronomic performance

Introduction

It is well known that the high content of linolenic acid leads to flavor instability and undesirable cooking odor in soybean seed oil[1, 2]. To over come this problem, various studies are going on to improve the oil quality by reducing the linolenic acid content in the oil. As a result, number of low linolenic acid germplasms have been developed by the use of X-ray irradiation and following hybridization of those mutant lines. LOLL, MS382, MOLL, M-5, M24 and KL-8 are the low linolenic acid lines developed from cultivar Bay. These lines have different levels of linolenic acid content, 2.8, 3.0, 3.4, 4.5, 5.9 and 6.6%, respectively, while Bay has 8.2 %[3, 4, 5, 6, 7]. Inheritance studies showed that, at least two major genes and modified genes are governed the low linolenic acid contents in these lines [3, 5, 6, 7].

However, to use these low linolenic acid lines for commercial production, it is neces-

sary to evaluate their agronomic performances and oil content, which should be in an optimum condition for profitable cultivation. Generally, mutations help to make genetic alterations by changing chromosome structure of the plant. Therefore, there is a possibility to make undesirable effect while changing linolenic acid content by irradiation. However, it depend on the association of different genes and overall mutational effect. Mutation can leads to negative effect, if mutated gene has pleiotropic effect, mutant allele link with undesirable allele at other loci or there are adverse effects due to physiological association of two traits [9].

Brossman and Wilcox[10] studied about genetic correlations of fatty acid composition of the oil and the agronomic traits of soybean, and concluded that there are few significant interrelations exist between them. Further, Wilcox et al.[11] investigated the relationship of low linolenic acid allele, *fan* (C1640) with agronomic traits and observed that there are no associations between them. In contrast, there are evidences to consider that affecting alleles which change the fatty acid compositions have been associated with other important traits. Lundeen et al.[12] identified in high stearic acid mutant A6, which has fas^a allele was associated with low yield, shorter plant height, early maturing and more lodging.

The objectives of this study were to evaluate the agronomic performances and oil content of lines LOLL, MS382, MOLL, M-5, M24 and KL-8 while altering its linolenic acid content in the seed oil, and compare its performances with original cultivar Bay.

Materials and Methods

Six low linolenic acid soybean lines and cultivar Bay were used as the experimental materials for this study. Bay is the original cultivar which used to develop these lines. M -5, M24 and KL-8 are the mutational derivatives of Bay. Each contains about 4.5, 5.9 and 6.6% linolenic acid, respectively. MS382 is a re-irradiated product of M-5 and contains about 3.0% linolenic acid. LOLL and MOLL are the recombinant low linolenic acid lines of M-5 x M24 and M-5 x KL-8 crosses, respectively and contain about 2.8 and 3.5 % linolenic acid, respectively.

Due to limited number of seeds, seeds were sterilized with fungicide and each seed was sown in a small plastic pot with fresh soil in glass house at Saga University, on 20 July 1996. After ten days seedlings were transplanted in the field. Experiment was conducted in a randomized complete block design with four replications. Each plot consisted five rows with 2m long planted 60 cm apart and the space between plants was 15 cm. Standard cultural practices were followed. Field irrigation, weed and insects control were done as needed.

Flowering time was recorded in each plant as the date of first flower appearance. The date, about 95 % of pods getting brown color was considered as maturity date. Days of maturity was recorded in each plant from the sowing. At maturity, boarder plants were removed and randomly selected eight plants from each plot were used to analyze the agromonic traits. Plant heights were measured in cm, from soil surface to tip of the main stem. Number of branches, number of main stem nodes, number of pods and total seeds

per plant were counted. Pods only with filled grains were counted. Rest of the plants in each plot were harvested and threshed separately. After getting agronomic data, those seeds were combined with respective plot and seed yields were measured in g m^{-2}.

Oil content and oil composition were analyzed using randomly selected seed sample from each plot. Oil content was analyzed by Soxhlet method and fatty acid composition was determined using gas chromatography, described by Takagi et al.[13].

Analysis of variance was computed for all the data. Duncan's multiple range test was used for mean comparison. Phenotypic correlation coefficients between linolenic acid content, agronomic traits and oil content were calculated.

Results and Discussion

The mean values of flowering date, maturity date, plant height, number of branches, number of nodes, total pods, total seeds, seed yield and oil content of each line and cultivar Bay are summarized in Table 1. It was observed that all the lines came to flowering within 5 days, starting from 24 August. Bay is a determinate type cultivar, and under the normal conditions it takes about 30-35 days from germination to flowering. Similar to this, the time of maturity of these lines occurred within 5 days. These results showed that, there are no significant differences between lines and its original cultivar Bay for the time of flowering and maturity.

Significant differences were observed in plant height, number of branches and number of nodes of these lines. Bay showed the tallest plants with the mean of 40.9cm. The plant height of M24 was comparable to Bay. The mean plant height of M-5 and MS382 were 36. 9 and 35.8 cm, respectively and no significant difference was observed between them. LOLL was next to M-5 and MS382 with the mean of 33.1 cm. MOLL and KL-8 showed the shortest plants with the mean of 26.4 and 30. 1 cm, respectively. LOLL, MS382, M-5, M24 and Bay showed similar number of branches consisted with 4-5 branches per plant. MOLL and KL-8 showed comparably fewer number of branches or in some cases no branches at

Table 1 Mean values of agronomic performances and oil content of low linolenic acid lines and cultivar Bay.

Line	Flowering†	Maturity‡	Plant height	Branches	Nodes	Tatal pods	Total seeds	Yield	Oil content
	date		cm	no. plant^{-1}				g m^{-2}	%
LOLL	24	29	33.1c*	4.9ab	11.6a	66.7b	140.0b	203.4bc	19.9
MS382	26	33	35.8b	4.0b	11.1a	60.0c	121.5c	190.0c	19.1
MOLL	26	30	30.1b	1.2c	9.9b	13.5d	25.5d	34.8d	18.8
M-5	26	30	36.9b	4.6ab	11.5a	71.6ab	144.1b	218.2b	18.6
M24	28	33	39.9a	5.2a	11.3a	68.3b	144.1b	224.8ab	19.4
KL-8	25	30	26.4e	0.1d	10.0b	11.8d	25.3d	33.6d	18.8
Bay	25	31	40.9a	5.4a	11.6a	75.7a	163.2a	241.0a	19.3

† : Flowering date was expressed in days after July 31st
‡ : Maturity date was expressed in days after September 30th
* : Values followed by the same letters are not significantly different at 0.05 probability level, acoording to the Duncan's multiple range test.

all. Similar results were observed in number of nodes, with the average of 11-12 nodes per plant. Except MOLL and KL-8, among the other lines no significant differences were observed.

Regarding the yield characters, number of pods per plant, number of seeds per plant and seed yield were evaluated. According to the statistical analysis, significant variability was observed in number of pods per plant. Bay showed the highest number of pods with the mean of 76 pods per plant. The number of pods in LOLL, M-5, and M24 were not significantly different from Bay. Pod number of MS382 was next to those. MOLL and KL-8 showed the lowest pod yield with the mean of 12 and 14 pods per plant, respectively.

Number of seeds per plant significantly varied among these lines. The highest number of seeds were observed in Bay with the mean of 163 seeds. LOLL, M-5 and M24 showed the next highest number, and there was no significant difference between them. Mean seed number per plant in MS382 was 121.5. MOLL and KL-8 gave the lowest number, each with the mean of 25 seeds per plant. Number of seeds per pod was ranged from 2-3 in all the lines.

The mean yield of LOLL, MS382, MOLL, M-5, M24, KL-8 and Bay were 203.4, 190, 34.8, 218.2, 224.8, 33.6 and 241 g m^{-2}, respectively. According to the statistical analysis, no significant difference was observed between M-5, M24, and Bay. Yield of LOLL and MS382 were significantly lower than Bay. Extremely poor yield was observed in MOLL and KL-8. Board[14] identified in the determinate type soybean plants, there is a high correlation of seed yield with the number of branches, number of nodes and number of pods. Similar correlation was observed in this study (Table 3). Further, this data showed that there were no significant variations in number of branches, number of nodes and number of pods per plant between Bay and the other lines, except MOLL and KL-8. It concludes that there is no big barrier to improve the yielding capacity of these lines as Bay.

Analytical data of seed oil content of these lines were ranged from 18.6 to 19.9%, and not significantly different from Bay which contained 19.3% oil (Table 1). Fatty acid composition of these lines showed that it was significantly varied among the lines (Table 2). The linolenic acid contents of LOLL, MS382, MOLL, M-5, M24 and KL-8 were 2.7, 2.

Table 2 Mean values of fatty acid composition in seed oil of low linolenic acid lines and cultivar Bay.

Line	Pamitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
			%		
LOLL	10.3b	2.8a	33.9a	50.2c	2.7f
MS382	10.8a	2.7a	33.7a	50.0c	2.7f
MOLL	9.0d	2.2b	33.8a	51.3bc	3.7e
M-5	10.8a	2.8a	30.0b	52.4abc	4.1d
M24	9.5c	2.5ab	28.5bc	53.6ab	5.8c
KL-8	9.3cd	2.9a	27.1c	54.3a	6.5b
Bay	10.6ab	2.7a	28.1bc	51.0bc	7.6a

Values followed by the same letters are not significantly different at 0.05 probability level, according to the Duncan's multiple range test.

7, 3.7, 4.1, 5.8 and 6.5%, respectively while in Bay it was 7.6%. No significant difference was observed between LOLL and MS382. These results agree with the early studies[3, 4, 5, 6, 7].

Evaluation study of agronomic performances of these lines showed that it varied from line to line. In line M-5, the reduction of linolenic acid is governed by the recessive allele, *fan* [8]. It contains 4.1% linolenic acid which is about half of that of Bay. Even though it showed a little shorter plant height, but number of nodes, number of branches and oil content were comparable to Bay. The yield was about 10% reduction from Bay. However, according to the statistical analysis this difference was not significant. The same *fan* allele with similar effect on linolenic acid was earlier identified in mutant C1640[15]. They studied about the relationship of this allele with agronomic traits and concluded that there are no associations between *fan* allele and agronomic traits[11]. Therefore, in line M-5, the little reduction of agronomic performances may be due to some effects which is independent from the mutation for linolenic acid.

Mutant KL-8 and its delivery MOLL showed distinctly inferior performances compare to the other lines. Inheritance studies indicated that the linolenic acid content in KL-8 was controlled by the modified allele, *fanx* on different locus from the *fan* allele in M-5 [5]. The allelic constituent for low linolenic acid in MOLL is *fanfanfanxfanx*[5]. It was observed that all the traits other than linolenic acid content in MOLL were similar to KL-8. It indicates that the inheritance of agronomic traits in KL-8 is dominant over M-5. Furthermore, at selection, it was unable to select the line with low linolenic acid content and good agronomic performances from the cross M-5 x KL-8. This may be due to detrimental effect of mutation or negative association of modified allele *fanx* with poor agronomic traits, such as pleiotropic effect or linkage. Additional studies are necessary to get clear about this.

The reduction of linolenic acid content in line M24 was due to involvement of different allele *fanx*^{a}, which was assumed as caused by a mutation at *Fanx* allele[6]. According to the data it is clear that the agronomic performances and oil content of this line is comparable to Bay and linolenic acid content is significantly lower than Bay. It indicates that the

Table 3 Phenotypic correlation coefficients of linolenic acid content, agronomic traits and oil content.

	Flowering	Maturity	Plant height	No. of branches	No. of nodes	Total pods	Total seeds	Yield	Oil content
Linolenic acid	0.108	0.204	0.216	−0.038	−0.039	−0.014	0.093	0.124	−0.123
Flowering		0.639	0.401	0.149	−0.036	0.100	0.168	0.217	−0.251
Maturity			0.542	0.274	0.141	0.276	0.366	0.363	−0.090
Plant height				0.909	0.814	0.888	0.932	0.941	0.306
No. of branches					0.965**	0.983*	0.971**	0.968**	0.557
No. of nodes						0.987**	0.962**	0.952**	0.541
Total pods							0.991**	0.984**	0.474
Total seeds								0.997**	0.414
Yield									0.395

*, ** : Significant at 0.05 and 0.01 probability levels, respectively.

reduction of linolenic acid by mutation in M24 is not negatively with the agronomic traits.

LOLL is a successful line which has about 2.7% linolenic acid content with comparable oil content as Bay. Its yield was about 15% reduction from Bay and little lower than both parents, M-5 and M24. The allelic constituent for low linolenic acid in LOLL is *fanfanfanx*^{a} *fanx*^{a} [6]. Even though it showed the little lower yield than both parents, statistically it was not significantly different. Number of branches, nodes, pods and seeds per plant were comparable to both parents.

The re-irradiated line MS382 contains about 2.7% linolenic acid and comparable oil content as Bay. However, low yielding capacity which is about 20% reduction from Bay, drove back its better performances. It was observed that the plant height, number of branches and number of nodes were similar to original line M-5 and yield was about 10% reduction from line M-5. The study of genetic behavior of MS382 suggested that the allelic constituent for low linolenic acid is similar to *fanfanfanx*^{a}*fanx*^{a} [7]. Since *fan* and *fanx*^{a} alleles were not shown any negative association with agronomic performances, the reduction of yield in LOLL and MS382 may independent from the mutation of linolenic acid.

Phenotypic correlation coefficients of linolenic acid content with agronomic traits and oil content are shown in Table 3. No significant correlation coefficient was observed between linolenic acid content and flowering time. However, previous literature showed that other than genetic involvement, there is an inverse relationship between linolenic acid content and environmental temperature at grain filling stage[16]. In this study, small variation of flowering time between these lines was not followed by a difference of environmental temperature at grain filling stage. Hence, it was not affected to make significant variation of linolenic acid content in these lines. It proves that the differences of linolenic acid content in these lines are only due to genetic variation. No significant correlation coefficients were observed between linolenic acid content and time of maturity, plant height, number of branches, number of nodes, number of pods, number of seeds per plant, seed yield and oil content. The lack of association between linolenic acid content and yield shows that there is a possibility to improve the yield while keeping low linolenic acid content in the seed oil.

According to the performances of LOLL, MS382, M-5 and M24, it was not shown any association between low linolenic acid allels *fan* and *fanx*^{a}, and alleles of agronomic traits. It indicates that there is a possibility to improve the agronomic and yield traits of these lines through back crossing. Furthermore, this study concludes that the mutational effect is not adversely effect on oil content, and no association was indicated between mutated alleles and the content of oil.

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ダイズBay品種より得られた低リノレン酸突然変異系統の農業形質

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適　　要

リノレン酸含量が，2.7%，2.7%，3.7%，4.1%，5.8%，6.5%，7.6%となるLOLL，MS382，MOLL，M-5，M24，KL-8，Bayの7系統について，リノレン酸含量の低下と各農業形質との関係について調査した．栽培は4区制の完全無作為法で行った．収穫後，各系統の開花期，成熟期，草丈，分枝数，主茎節数，総莢数，収量，脂質含量，脂肪酸組成の各農業形質について調べたところ，全ての系統でリノレン酸含量の低下と開花期，成熟期，脂質含量との間に，LOLL，MS382，M-5，M24，Bayではリノレン酸含量の低下と分枝数，主茎節数との間に、M-5，M24，Bayの間ではリノレン酸含量の低下と草丈，収量について差が認められなかった．KL-8とMOLL（MOLLはKL-8とM-5の交雑から得られた低リノレン酸系統）は他系統に較べてかなり劣る農業形質を示したことから，KL-8では低リノレン酸含量となる突然変異に加えて劣悪な農業形質の同時の突然変異があったものと考えた．残る他の系統について，LOLL，MS382，M-5，M24では，低リノレン酸含量は他の農業形質に影響することなく，両者の間の表現型相関は全く認められなかった．